Investigating the systems biology of aging in *Caenorhabditis elegans* at cellular resolution

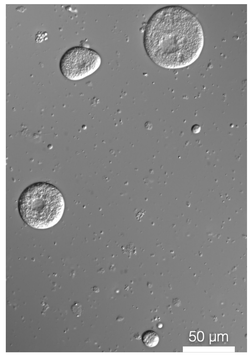
Erik Segerdell[1], Christine Sedore[1], Anna Coleman-Hulbert[1], Stephen Banse[1], Dhaval S. Patel[2], Hang Lu[2], Patrick Phillips[1]

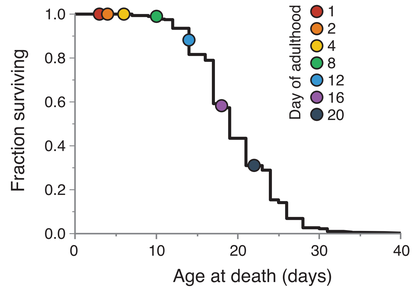
[1]Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA
[2]School of Chemical & Biomolecular Engineering, Georgia Institute of Technology, Atlanta, GA, USA

UNIVERSITY OF OREGON

Introduction

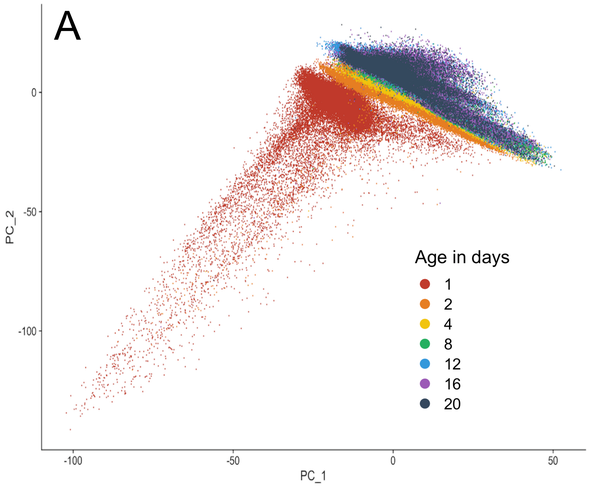
While the age-dependent prevalence of disease is one of humanity's most important health concerns, the underlying biological cause of aging has proven difficult to determine. From a systems biology perspective, aging can be explained as a degradation in complex functional regulatory networks caused by either changes in a limited set of central components, or by heterogeneous failure across the network, ultimately resulting in failure upon crossing a critical frailty threshold. *Caenorhabditis elegans*, a free-living nematode worm found in temperate soil environments, is an ideal model for systems-biology studies at the cellular, tissue, and inter-tissue levels. *C. elegans* has a short lifespan amenable to aging studies, and a precisely described cellular lineage and anatomy. We measured cellular heterogeneity in gene expression in *C. elegans* by profiling the transcriptomes of individual cells using single cell RNA-seq (scRNA-seq). scRNA-seq is a promising technology for investigating age-specific changes in gene regulatory networks at a cellular resolution.





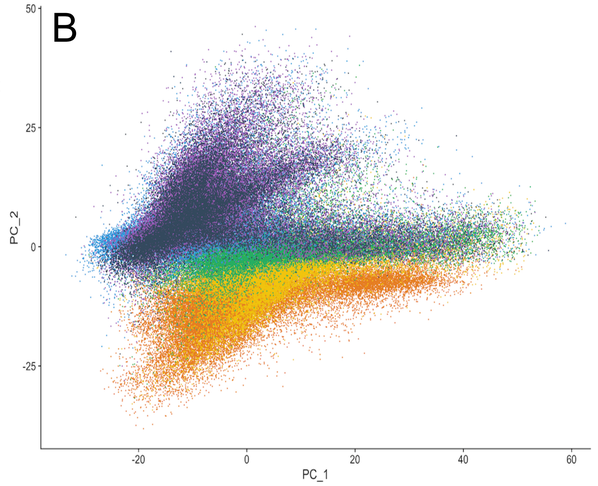
We have developed a pipeline to collect a complete dataset from cohorts of aging worms across their lifespan (3 independent replicates at each of 7 ages).

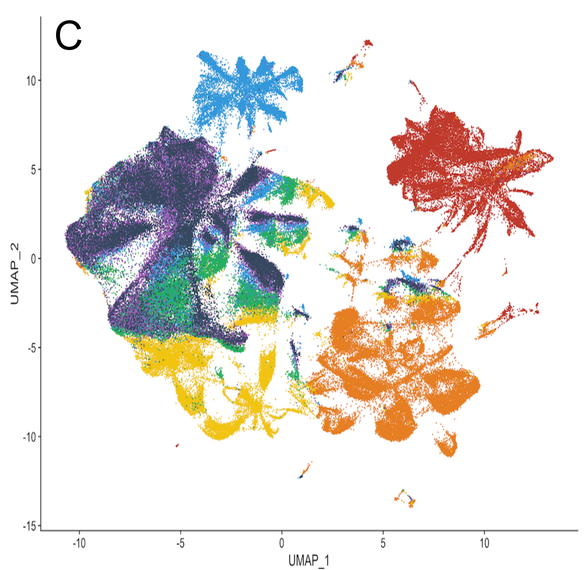
Initial findings



(A) Day 1 samples cluster away from all other ages, with more variance between day 1 samples being apparent relative to the within-day sample variance at later ages.

(B) Samples from day 2-onward show more within-day consistency while exhibiting a distinct aging progression from days 2-12, after which a more consistent aged state is maintained (unpublished data).

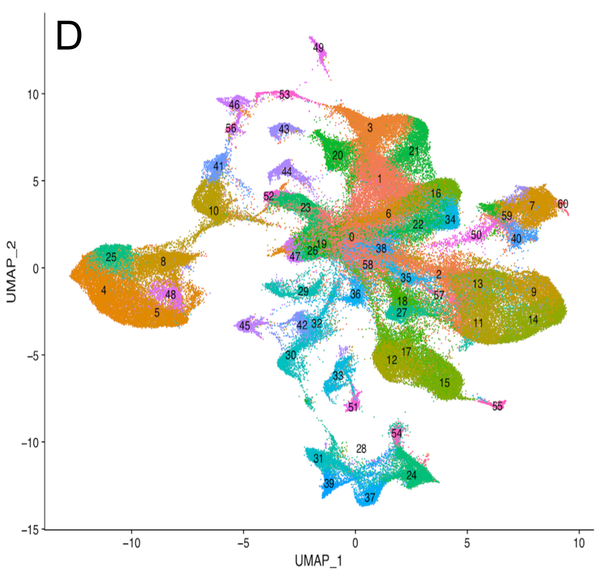




(C) Age progressions are visible in several clusters at the center of the UMAP projection of the whole combined dataset.

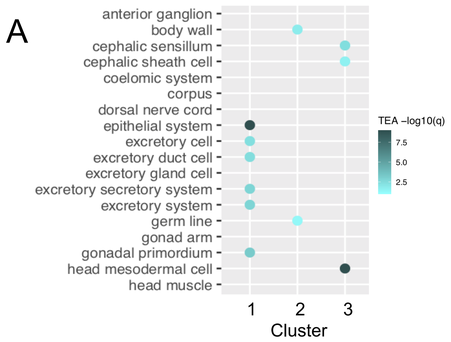
(D) For cluster identification we merged the day 2-20 samples and normalized the dataset using an integration workflow in the Seurat R toolkit based on the SCTransform modeling framework.

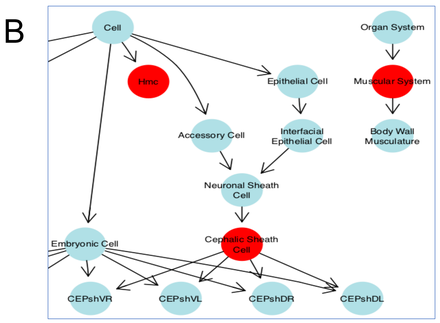
The integrated dataset comprises approximately 140,000 cells.



Cell identity and analysis

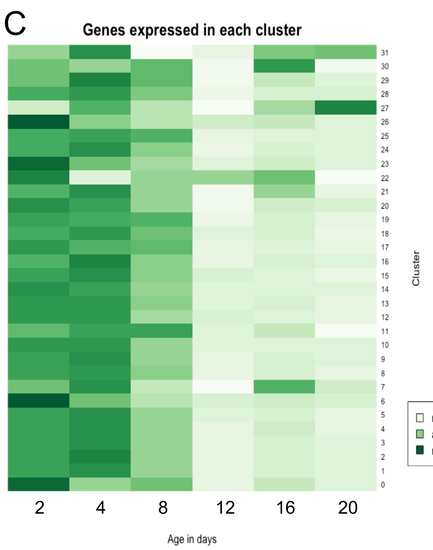
Cell and tissue identity were putatively assigned to clusters using an automated tissue enrichment analysis (TEA) workflow based on WormBase expression data.

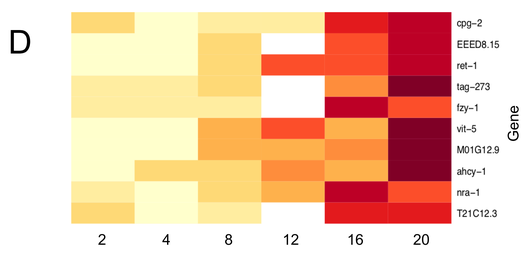


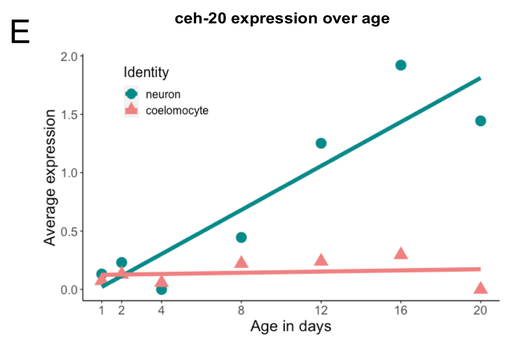


Enriched tissues in each set of cluster markers were identified at every clustering resolution and are visualized **(A)** in dotplots (blue plot points; only a part of the plot shown) and **(B)** within the *C. elegans* Anatomy Ontology hierarchy (red-filled nodes).

(C) Fewer genes per cluster are expressed at later ages as cluster identity seems to lose specificity.







To address the central aging question, we are analyzing changes in **(D)** gene variance and **(E)** expression at the tissue level as worms age.

Investigating trends at the cluster level, such as expression of aging-related transcription factor *ceh-20* in two different cell types, allows us to detect changes that are often masked in traditional whole-worm RNAseq.

Acknowledgments

John Willis, Phillips Lab
Zach Stevenson, Phillips Lab
Maggie Weitzman, GC3F

This research is supported by the National Institute on Aging of the National Institutes of Health under project number **5R01AG056436-03.**

THE INSTITUTE OF ECOLOGY AND EVOLUTION